Susceptibility of Pest *Nezara viridula* (Heteroptera: Pentatomidae) and Parasitoid *Trichopoda pennipes* (Diptera: Tachinidae) to Selected Insecticides

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ABSTRACT Susceptibility of the southern green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae), and its endoparasitoid *Trichopoda pennipes* (F.) (Diptera: Tachinidae) to acetamiprid, cyfluthrin, dicrotophos, indoxacarb, oxamyl, and thiamethoxam was compared in residual and oral toxicity tests. In the residual toxicity test, cyfluthrin, dicrotophos, and oxamyl were highly toxic to *N. viridula*. Thiamethoxam was moderately toxic to these insects. Each of the four insecticides was highly toxic to *T. pennipes* after prolonged tarsal contact with dried residues of these chemicals. In the oral toxicity test, where *N. viridula* fed on food covered with insecticide residues, none of the insecticides were toxic to adults of this stink bug, but acetamiprid, dicrotophos, and thiamethoxam were moderately toxic to the nymphs. In the oral toxicity test, where *N. viridula* fed on a gel-food containing insecticides, cyfluthrin, dicrotophos, oxamyl, and thiamethoxam were highly toxic to this stink bug. In an oral toxicity test using contaminated sugar water, all of the insecticides were highly toxic to *T. pennipes*. Because insecticides were as toxic, or more toxic, to *T. pennipes* than to *N. viridula*, it is extremely important to conserve this parasitoid by applying these insecticides for control of southern green stink bugs only when the pest reaches economic threshold.

KEY WORDS southern green stink bug, residual toxicity, oral toxicity

The southern green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae), is a widely distributed pest in tropical and subtropical regions of the world, and its host plant range encompasses >30 families of dicots, especially legumes, and a few monocot families, particularly grasses (Panizzi 1997). The pest is most attracted to fruit and pods of host plants, including field crops such as corn, soybean, cotton, and sorghum, and it feeds on their fruiting structures, causing economic damage to the crops (McPherson and McPherson 2000). As an example, total losses during 2004 for stink bugs, including *N. viridula; Acrosternum hilare* (Say), the green stink bug; and *Euschistus servus* (Say), the brown stink bug, were estimated at $9.7 million for the cotton industry across the United States (Williams 2005).

Trichopoda pennipes (F.) (Diptera: Tachinidae), an endoparasitoid of *N. viridula* nymphs and adults, is one of the most successful natural enemies of this pest (Todd and Lewis 1976, Buschman and Whitcomb 1980, McPherson et al. 1982, Temerak and Whitcomb 1984, Menezes et al. 1985, Jones 1988). Thus, insecticide selectivity to this natural enemy is an important issue in integrated pest management of *N. viridula.*

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The organophosphate dicrotophos is the standard insecticide used for control of *N. viridula, A. hilare,* and *E. servus* in cotton (*Gossypium* spp.). The carbamate oxamyl also has been used for control of *N. viridula* in cotton and soybean, *Glycine max* (L.) Merr. (Greene et al. 2003; Willrich et al. 2003, 2004b). The pyrethroids cyfluthrin, bifenthrin, zetacypermethrin, and lambda-cyhalothrin can provide control for stink bugs, although susceptibility to pyrethroids, except for bifenthrin, is higher for *N. viridula* and *A. hilare* than for *E. servus* (Emfinger et al. 2001, Greene et al. 2001). Toxicity of pyrethroids to *N. viridula* has been demonstrated in topical and residual studies in the field and laboratory (Greene et al. 2001, 2003; Greene and Capps 2003; Willrich et al. 2004a).

Indoxacarb, acetamiprid, and thiamethoxam are three new compounds that target plant-feeding pests. Indoxacarb is a new oxadiazine insecticide that is active on foliar feeding lepidopteran larvae (Wing et al. 2000). When the larvae ingest sprayed foliage or are sprayed directly, they stop feeding and either go into mild convulsions or a passive paralysis from which there is no recovery. Acetamiprid is a new systemic insecticide belonging to the neonicotinoid family. It acts on the central and peripheral nervous system, causing irreversible blocking of the postsynaptic nicotinergic acetylcholine receptors, which results in excitation and paralysis, followed by death (Bai et al.

1991). This insecticide has excellent systemic and translaminar properties and high residual activity (Takahashi et al. 1992); thus, it is particularly effective in controlling small plant-sucking pests such as whiteflies, aphids, and plant bugs. Thiamethoxam is a second generation neonicotinoid compound with contact and ingestion activity for many important sucking pests such as whiteflies, aphids, thrips, and plant bugs (Mason et al. 2000). Like acetamiprid, it interferes with the nicotinic acetylcholine receptors in the nervous system of the insect (Maienfisch et al. 2001). After foliar, soil application, or as a seed treatment, it has systemic and long residual activity.

Three general techniques are used for determining the toxicity of insecticides to natural enemies: 1) topical application of the insecticide on the insect, 2) exposure of the insect to dried residues of the insecticide applied to a substrate or plant part, and 3) monitoring natural enemy populations before and after applications of insecticides in the field. All three techniques provide valuable information on expected and observed impact of these insecticides on natural enemies in the field. Because novel insecticides with ingestion activity have been discovered, techniques need to be designed to determine the toxicity of an insecticide ingested by an insect. Many studies have used the first three methods to determine the effect of insecticides on various species of stink bug pests (Emfinger et al. 2001; Greene et al. 2001, 2003; Fitzpatrick et al. 2002a,b; Greene and Capps 2003; López et al. 2003; Willrich et al. 2004a,b). Only two studies have examined the effect of feeding on insecticides on pest stink bugs (Tillman and Mullinix 2004, Vandekerkhove and De Clercq 2004), and no studies have been conducted on the impact of insecticides on *T. pennipes.* Consequently, the goal for this research was to examine the susceptibility of *N. viridula* and *T. pennipes* to acetamiprid, cyfluthrin, dicrotophos, indoxacarb, oxamyl, and thiamethoxam in both residual and oral toxicity tests.

Materials and Methods

Insects. *N. viridula* adults were collected from sorghum, *Sorghum bicolor* (L.) Moench, in Tift County, Georgia, in 2004 and then held in a room at 24–27°C and 40–50% RH. Many of these field-collected adults were parasitized by *T. pennipes.* After collection, all the stink bugs were put in a cage (29.85 by 29.85 by 29.85 cm^3) with a hardware cloth screen (3.18-mm mesh) on the bottom. Last instars of *T. pennipes* dropped through the screen unto a tray underneath the cage, and then they pupated. Tachinid pupae then were removed from the cage and held for emergence of adults. Unparasitized stink bug adults were used to establish a colony of *N. viridula.* Stink bugs were fed raw, shelled peanuts and pole beans *Phaseolus vulgaris* L. *T. pennipes* adults were fed sugar water (300 g of granulated sugar and 5 g of ascorbic acid in 3.8 liters of distilled water). First generation third instars ($\approx$3 to 4 d after ecdysis), 7- to 9-d-old females of *N. viridula,* and 2- to 3-d-old *T. pennipes* adults were used in toxicity tests. Voucher specimens of these insects are held in the USDA–ARS, Crop Protection and Management Research Laboratory in Tifton, GA.

Insecticides. Doses of each insecticide used in these experiments simulated the concentrations of field-use rates based on applications at a total volume of 93.5 liters/ha. The test included the following six treatments and rates: 1) acetamiprid (Assail 70 [577 μg/ml], Cerexagri, Inc., King of Prussia, PA), 2) cyfluthrin (Baythroid 2 [346 μg/ml], Bayer Corp., Atlanta, GA), 3) dicrotophos (Bidrin 8 [3,806 μg/ml], Amvac, Los Angeles, CA), 4) indoxacarb (Steward 1.25 [1,038 μg/ml], DuPont, Wilmington, DE), 5) oxamyl (Vydate 2 [2,883 μg/ml], DuPont), and 6) thiamethoxam (Centric 25 [715 μg/ml], Syngenta, Greensboro, NC).

Residual Toxicity. In residual tests, an insecticide treatment was sprayed on the top and bottom of a plastic petri dish (100 by 15 mm) by using a Preval sprayer (Precision Valve Corp., Yonkers, NY). Water was used as the control. After the compounds were allowed to dry for 1 h, *N. viridula* nymphs were placed singly in the petri dishes. A randomized complete block design was used with five insects per block (day) for eight blocks (40 insects per treatment). Insects were not fed during the exposure period to avoid the possibility of the insects feeding on contaminated food. After 24 h, insects were moved to clean petri dishes and provided food and water. The whole procedure was repeated for *N. viridula* adult females. The procedure was similar for *T. pennipes* adults, except a randomized complete block design was used with two pairs (each pair with one male and one female) of insects per block (day) for three blocks (12 insects per treatment). In all experiments, if insects were incapacitated, but able to right themselves when turned on their backs, they were considered to be "knocked down," but not dead. If insects were unable to right themselves when turned on their backs, they were considered to be dead. Knockdown was recorded 1 d after treatment, and mortality that occurred after the insects were observed to be knocked down was recorded 4 d after exposure to insecticide residues. Mortality that occurred without first knocking down the insects was recorded both 1 and 4 d after exposure to the insecticides.

Oral Toxicity. For *N. viridula,* oral toxicity was tested using two techniques. The first technique was used to determine the effect of stink bugs feeding on food covered with insecticide residues. Before the test, food (pole beans) was dipped and held in an insecticide treatment for 30 s and placed on a wire screen for 1 h to allow the chemical to dry. Water was used as the control. After the materials dried, treated food was wrapped in Parafilm (American National Can, Menasha, WI) to eliminate the possibility of the insects being exposed to residues of the compound while feeding. Then, *N. viridula* nymphs were placed singly in the petri dishes (100 by 15 mm) with treated food. A randomized complete block design was used with five insects per block for a total of eight blocks (40 insects per treatment). The whole procedure was repeated for *N. viridula* adult females.

A second technique was used to determine the effect on *N. viridula* feeding on gel-like food contaminated with an insecticide. Stink bugs drink free water even when food is available. In the field, these bugs can be exposed to insecticides through contaminated water. Also, some insecticides have systemic activity, thus the stink bugs can be exposed to these insecticides by feeding on plant tissue of insecticide-treated plants. Before the test, a bean solution was made by blending 85 g of whole pole beans in 250 ml of water and then straining the mixture with cheese cloth. The insecticide concentrations mentioned above were prepared using this bean solution instead of water. The cap from a 1.5-mm centrifuge micro tube (Fisher, Suwanee, GA) was used as a feeding well for stink bugs, and 0.005 g of Water Lock Super Absorbent Polymer (Grain Processing Corp., Muscatine, IA) was placed in the bottom of this well. By using a disposable transfer pipet, 0.27 ml of insecticide solution (or bean solution only for the control) was dropped into this well. The solution was allowed to gel, and then a piece of Parafilm was stretched over the top of the well, leaving a narrow opening on a side of the well to allow the stink bugs to feed on the gel-like "food." Feeding wells were placed singly in petri dishes (35 by 10 mm). Then, *N. viridula* nymphs were placed singly in the petri dishes. A randomized complete block design was used with two insects per block for a total of eight blocks (16 insects per treatment). The whole procedure was repeated for *N. viridula* adult females.

In the field, *T. pennipes* adults drink free water and feed on extrafloral nectaries. Thus, for *T. pennipes* adults, oral toxicity was tested by allowing insects to feed on insecticide-treated sugar water. Before the test, ≈0.3 ml of treated food was dropped into a cap of a 1.5-mm centrifuge micro tube by using a disposable transfer pipet. Sugar water was used as the control. Parafilm was stretched over the top of the cap to eliminate exposure of insects to residues of the compound while feeding, but an ≈0.2-cm narrow opening was left on one side of the cap to facilitate feeding by the fly. These centrifuge caps with insecticide-treated sugar water are referred to as feeding wells hereafter. After individual feeding wells were placed in petri dishes (60 by 15 mm), *T. pennipes* adults were placed singly into these feeding arenas. A randomized complete block design was used with two pairs (each pair with one male and one female) of insects per block (day) for three blocks (12 insects per treatment).

For both species, insects were starved 24 h before feeding tests. During the test, insects were allowed to feed once, and feeding time was recorded. After the insects fed on treated food, they were placed individually in clean petri dishes and given insecticide-free pole beans or sugar water, depending on the insect species. If insects became immobile during feeding they were considered to be "knocked out," and the amount of time the insects were knocked out was recorded. Mortality was recorded at one and 4 d after treatment for tests where insects fed on insecticide-treated food. In tests in which insects fed on contaminated food, mortality occurred relatively quickly, so mortality data were recorded at 2 d after treatment.

Statistical Analysis. For *N. viridula* in residual toxicity tests, preliminary analyses of mortality showed that variances between nymphs and adults were unequal. Therefore, these data were analyzed separately for developmental stages and days after treatment using PROC MIXED (SAS Institute 1999). The fixed effect was insecticide. Random effects were block, block by insecticide, and residual error. Knockdown data were analyzed using PROC MIXED (SAS Institute 1999) with the same model. Because knockdown occurred only for *N. viridula* females exposed to cyfluthrin, acetamiprid, and thiamethoxam residues, data on percentage of mortality resulting from knockdown were analyzed for only these three treatments by using PROC MIXED (SAS Institute 1999) with the abovementioned model. Least squares means were separated by least significant difference (LSD) (SAS Institute 1999) where appropriate. Means for percentage of mortality for developmental stages and days after treatment were compared using Student's *t*-tests. Means for percentage of knockdown and percentage of mortality resulting from knockdown were compared using Student's *t*-tests.

For *T. pennipes* in residual toxicity tests, preliminary analyses of mortality data showed that there was a significant insecticide by day interaction. Therefore, evaluations were conducted for each day after treatment. Percentage of mortality data were analyzed using PROC MIXED (SAS Institute 1999). The fixed effects were insecticide, sex, and insecticide by sex. Random effects were block, block by insecticide, block by sex, block by sex within insecticide, and residual error. Least squares means were separated by LSD (SAS Institute 1999) where appropriate. Means for percentage of mortality for days after treatment were compared using Student's *t*-tests.

In oral toxicity tests for *N. viridula*, preliminary analyses of mortality, feeding time, and knockout time data showed that variances between nymphs and adults were unequal. Therefore, these data were analyzed separately for developmental stages and days after treatment using PROC MIXED (SAS Institute 1999). The fixed effect was insecticide. Random effects were block, block by insecticide, and residual error. Least squares means were separated by LSD (SAS Institute 1999) where appropriate. Means for percentage of mortality, feeding time, and knockout time for developmental stages and days after treatment were compared using Student's *t*-tests.

For *T. pennipes* in oral toxicity tests, feeding time and mortality data were analyzed using PROC MIXED (SAS Institute 1999). The fixed effects were insecticide, sex, and insecticide by sex. Random effects were block, block by insecticide, pair number within block by treatment, block by sex, insecticide by sex, and residual error. Least squares means were separated by LSD (SAS Institute 1999) where appropriate. Preliminary analyses of feeding time data showed that there was a significant insecticide by sex interaction. Closer examination of means revealed that the difference

Table 1. Least squares means for percentage of mortality 1 and 4 d after treatment for *N. viridula* nymphs and adults and for percentage knockdown 1 d after treatment and percentage mortality after knockdown for adults exposed to residues of acetamiprid, cyfluthrin, dicrotophos, indoxacarb, oxamyl, and thiamethoxam

Insecticide	μg/ml	% mortality 1 d after treatment		% mortality 4 d after treatment		% knockdown 1 d after treatment	% mortality after knockdown[a]
		Nymphs	Adults	Nymphs	Adults	Adults	Adults
Acetamiprid	577	100.0a1A	47.5b1B	100.0a1A	55.0c1B	32.5ab1	5.0b2
Cyfluthrin	346	100.0a1A	52.5b2B	100.0a1A	100.0a1A	47.5a1	47.5a1
Dicrotophos	3,806	100.0a1A	100.0a1A	100.0a1A	100.0a1A	0c	
Indoxacarb	1,038	0c1A	0c1A	2.5c1A	0d1A	0c	
Oxamyl	2,883	100.0a1A	100.0a1A	100.0a1A	100.0a1A	0c	
Thiamethoxam	715	82.5b1A	80.0a1A	85.0b1A	80.0b1A	15.0bc1	0b1
Control		2.5c1A	0c1A	2.5c1A	0d1A	0c	

For mortality data, least squares means within a column followed by the same lowercase letter are not significantly different between insecticides at 1 d after treatment for nymphs (PROC MIXED, LSD, df = 21, $P > 0.05$, SE = 4.23, $n = 40$) and adults (PROC MIXED, LSD, df = 21, $P > 0.05$, SE = 11.7, $n = 40$). Least squares means within a column followed by the same lowercase letter are not significantly different between insecticides at 4 d after treatment for nymphs (PROC MIXED, LSD, df = 21, $P > 0.05$, SE = 5.0, $n = 40$) and adults (PROC MIXED, LSD, df = 21, $P > 0.05$, SE = 9.13, $n = 40$). Means within a row followed by the same number are not significantly different between days after treatment for a single insecticide for nymphs (Student's t-test, df = 42, $P > 0.05$, SE = 4.64, $n = 40$) and adults (Student's t-test, df = 42, $P > 0.05$, SE = 10.51, $n = 40$). Means within a row followed by the same capital letter are not significantly different between developmental stages for a single insecticide at 1 d after treatment (Student's t-test, df = 42, $P > 0.05$, SE = 9.3, $n = 40$) and at 4 d after treatment (Student's t-test, df = 42, $P > 0.05$, SE = 7.37, $n = 40$). For knockdown data, least squares means within a column followed by the same lowercase letter are not significantly different between insecticides (PROC MIXED, LSD, df = 21, $P > 0.05$, SE = 10.52, $n = 40$). For mortality occurring after knockdown, least squares means within a column followed by the same lowercase letter are not significantly different between insecticides (PROC MIXED, LSD, df = 9, $P > 0.05$, SE = 12.16, $n = 40$). Means within a row followed by the same no. are not significantly different between percentage of knockdown and percentage of mortality resulting from knockdown (Student's t-test, df = 18, $P > 0.1$, SE = 14.26, $n = 40$).

[a] At 4 d after treatment.

between males and females in controls was large in comparison to the difference between the sexes for all other treatments. Therefore, analysis of feeding time was run without the control.

Results

Residual Toxicity. In residual toxicity tests with *N. viridula,* a significant insecticide effect was detected for percentage of mortality data 1 d after treatment for third instars ($F = 246.58$; df = 6, 21; $P = 0.0001$) and adult females ($F = 26.26$; df = 6, 21; $P = 0.0001$) and 4 d after treatment for third instars ($F = 172.53$; df = 6, 21; $P = 0.0001$) and adult females ($F = 49.58$; df = 6, 21; $P = 0.0001$). Overall, residues of dicrotophos, oxamyl, and cyfluthrin were highly toxic; thiamethoxam was moderately toxic; and indoxacarb was nontoxic to *N. viridula* (Table 1). Residues of acetamiprid were highly toxic to nymphs but only moderately toxic to adults. Percentage of mortality was significantly higher for adults exposed to residues of cyfluthrin at 4 d after treatment compared with 1 d after treatment, suggesting that this insecticide killed the insects slowly. A significant change in percentage of mortality did not occur over time when adults were exposed to residues of dicrotophos, oxamyl, acetamiprid, and thiamethoxam indicating that these insecticides acted quickly.

A significant insecticide effect was detected for percentage of knockdown 1 d after treatment ($F = 6.77$; df = 6, 21; $P = 0.0004$) and for percentage of mortality after knockdown ($F = 12.21$; df = 2, 9; $P = 0.0129$) for *N. viridula* adults exposed to residues of insecticides. Knockdown 1 d after treatment only occurred for adults exposed to residues of cyfluthrin, acetamiprid, and thiamethoxam (Table 1). Percentage of knockdown was significantly higher for cyfluthrin and acetamiprid than for the control, whereas there was no significant difference in percentage of knockdown between thiamethoxam and the control. Percentage of mortality was significantly higher for cyfluthrin than for acetamiprid and thiamethoxam. Also, there was no significant difference in percentage of knockdown and percentage of mortality after knockdown for cyfluthrin. Therefore, for cyfluthrin, mortality generally followed knockdown, indicating again that it acts over time.

For *T. pennipes,* factorial analysis revealed that there was a significant insecticide effect for percentage of mortality data 1 d after treatment ($F = 9.65$; df = 6, 12; $P = 0.0005$) and 4 d after treatment ($F = 62.0$; df = 6, 56; $P = 0.0001$) in residual toxicity tests. There was no significant sex effect ($F = 0.25$; df = 1, 56; $P = 0.619$) and insecticide and sex interaction ($F = 0.25$; df = 6, 56; $P = 0.9573$) for percentage of mortality data 1 d after treatment, and there was also no significant sex effect ($F = 0$; df = 1, 56; $P = 1.0$) and insecticide and sex interaction ($F = 0.67$; df = 6, 56; $P = 0.6768$) for percentage of mortality data 4 d after treatment. Therefore, the four insects (two males and two females) in a block were considered to be four random samples. Overall, regarding residues, dicrotophos, oxamyl, cyfluthrin, and thiamethoxam were highly toxic; acetamiprid was moderately toxic; and indoxacarb was nontoxic to *T. pennipes* adults (Table 2). Cyfluthrin, thiamethoxam, and acetamiprid caused significantly higher mortality 4 d after treatment compared with 1 d after treatment, suggesting that these insecticides acted slowly. Tarsal exposure to insecticide residues

Table 2. Least squares means for percentage of mortality 1 and 4 d after treatment for *T. pennipes* adults exposed to residues of acetamiprid, cyfluthrin, dicrotophos, indoxacarb, oxamyl, and thiamethoxam

Insecticide	μg/ml	% mortality 1 d after treatment	% mortality 4 d after treatment
Acetamiprid	577	16.67b2	75.0b1
Cyfluthrin	346	16.67b2	100.0a1
Dicrotophos	3,806	100.0a1	100.0a1
Indoxacarb	1,038	0b1	0c1
Oxamyl	2,883	100.0a1	100.0a1
Thiamethoxam	715	41.67b2	91.67ab1
Control		0b1	0c1

Least squares means within a column followed by the same lowercase letter are not significantly different between insecticides for mortality 1 d after treatment (PROC MIXED, LSD, df = 12, $P > 0.05$, SE = 19.92, $n = 12$). Least squares means within a column followed by the same lowercase letter are not significantly different between insecticides for mortality 4 d after treatment (PROC MIXED, LSD, df = 12, $P > 0.05$, SE = 8.33, $n = 12$). Means within a row followed by the same no. are not significantly different between percentage of mortality at 1 d after treatment and percentage of mortality at 4 d after treatment (Student's *t*-test, df = 24, $P > 0.1$, SE = 15.28, $n = 40$.

did not knockdown *T. pennipes* adults any time during the test.

Oral Toxicity. For *N. viridula*, a significant insecticide effect was detected for percentage of mortality data 1 d after treatment for third instars ($F = 12.5$; df = 6, 18; $P = 0.0001$) and adult females ($F = 5.01$; df = 6, 270; $P = 0.0001$) and 4 d after treatment for third instars ($F = 17.27$; df = 6, 18; $P = 0.0001$) and adult females ($F = 6.91$; df = 6, 273; $P = 0.0001$) feeding on pole beans covered with insecticide residues. Overall, dicrotophos, thiamethoxam, and acetamiprid were moderately toxic and cyfluthrin, oxamyl, and indoxacarb were nontoxic to *N. viridula* nymphs in these tests (Table 3). Thiamethoxam and dicrotophos were only slightly toxic, and acetamiprid, cyfluthrin, oxamyl, and indoxacarb were nontoxic to *N. viridula* adults. Dicrotophos, thiamethoxam, and acetamiprid were significantly more toxic to nymphs compared with adults. For thiamethoxam and acetamiprid, mortality was significantly higher 4 d after treatment compared with 1 d after treatment for nymphs. For adults, there was no additional mortality detected for dicrotophos and thiamethoxam from 1 to 4 d after treatment.

There was a significant insecticide effect for feeding time for third instars ($F = 39.64$; df = 6, 270; $P = 0.0001$) and adult females ($F = 16.06$; df = 6, 21; $P = 0.0001$) feeding on insecticide-treated pole beans compared with those feeding on untreated food. Feeding time was significantly reduced for both nymphs and adults in these tests (Table 4). Feeding time was significantly lower for nymphs and adults feeding on food with residues of acetamiprid, dicrotophos, and thiamethoxam than for those feeding on food with residues of cyfluthrin, indoxacarb, and oxamyl. Acetamiprid, dicrotophos, and thiamethoxam reduced feeding of nymphs and adults by ≈86–98% compared with the control, whereas cyfluthrin, indoxacarb, and oxamyl reduced feeding by ≈20–60% compared with the control. Except for cyfluthrin, feeding time was not significantly different between nymphs and adults. However, over all treatments, mean feeding time for adults (46.57 ± 4.15) was significantly lower than that for nymphs (66.66 ± 3.25) ($t = 3.82$, df = 12, SE = 5.28).

A significant insecticide effect was detected for knockout time after feeding on insecticide-treated poles beans for third instars ($F = 6.01$; df = 6, 18.4; $P = 0.0013$) and adult females ($F = 3.56$; df = 6, 21; $P = 0.0136$). There also was a significant insecticide effect for mortality after knockout at feeding for third instars ($F = 8.31$; df = 4, 12; $P = 0.0002$) and adult females ($F = 4.08$; df = 3, 156; $P = 0.0006$) in these oral toxicity tests. Knockout time was significantly higher for acetamiprid, dicrotophos, and thiamethoxam than for all other treatments for nymphs and subsequent mortality after knockout was higher for these treatments compared with all other treatments (Table 4). Knockout time was significantly higher for thiamethoxam than for all other treatments for adults, and percentage

Table 3. Least squares means for percentage of mortality 1 and 4 d after treatment for *N. viridula* nymphs and adults feeding on food with residues of acetamiprid, cyfluthrin, dicrotophos, indoxacarb, oxamyl, and thiamethoxam

Insecticide	μg/ml	% mortality 1 d after treatment		% mortality 4 d after treatment	
		Nymphs	Adults	Nymphs	Adults
Acetamiprid	577	27.5b2A	0c1B	45.0a1A	0c1B
Cyfluthrin	346	5.0c1A	2.5bc1A	5.0b1A	2.5c1A
Dicrotophos	3,806	50.0a1A	10.0ab1B	60.0a1A	12.5b1B
Indoxacarb	1,038	2.5c1A	0c1A	10.0b1A	0c1A
Oxamyl	2,883	0c1A	0c1A	0b1A	0c1A
Thiamethoxam	715	30.0b2A	17.5a1A	47.5a1A	22.5a1B
Control		0c1A	0c1A	0b1A	0c1A

Least squares means within a column followed by the same lowercase letter are not significantly different between insecticides at 1 d after treatment for nymphs (PROC MIXED, LSD, df = 18, $P > 0.05$, SE = 7.83, $n = 40$) and adults (PROC MIXED, LSD, df = 18, $P > 0.05$, SE = 4.35, $n = 40$). Least squares means within a column followed by the same lowercase letter are not significantly different between insecticides at 4 d after treatment for nymphs (PROC MIXED, LSD, df = 18, $P > 0.05$, SE = 8.79, $n = 40$) and adults (PROC MIXED, LSD, df = 18, $P > 0.05$, SE = 4.76, $n = 40$). Means within a row followed by the same no. are not significantly different between days after treatment for a single insecticide for nymphs (Student's *t*-test, df = 36, $P > 0.05$, SE = 8.33, $n = 40$) and adults (Student's *t*-test, df = 36, $P > 0.05$, SE = 4.56, $n = 40$). Means within a row followed by the same capital letter are not significantly different between developmental stages for a single insecticide at 1 d after treatment (Student's *t*-test, df= 36, $P > 0.05$, SE = 6.34, $n = 40$) and at 4 d after treatment (Student's *t*-test, df = 36, $P > 0.05$, SE = 6.47, $n = 40$).

Table 4. Least squares means for feeding time, knockout time at feeding, and percentage of mortality after knockout for *N. viridula* feeding on food with residues of acetamiprid, cyfluthrin, dicrotophos, indoxacarb, oxamyl, and thiamethoxam

Insecticide	μg/ml	Feeding time (s)		Knockout time at feeding (s)		% mortality after knockout at feeding	
		Nymphs	Adults	Nymphs	Adults	Nymphs	Adults
Acetamiprid	577	15.33e1	2.55d1	416.05a1	86.88b2	42.5a1	0b2
Cyfluthrin	346	119.2b1	49.35c2	72.0b1	36.0b1	5.0b1	2.5b1
Dicrotophos	3,806	20.38e1	12.6d1	576.0a1	76.38b2	40.0a1	7.5ab2
Indoxacarb	1,038	89.5c1	82.63b1	0b1	0b1		
Oxamyl	2,883	63.65d1	47.65c1	13.98b1	0b1	0b1	
Thiamethoxam	715	11.68e1	8.83d1	571.1a1	499.48a1	42.5a1	15.0a2
Control		146.85a1	122.35a1	0b1	0b1		

Least squares means within a column followed by the same lowercase letter are not significantly different between insecticides for feeding time for nymphs (PROC MIXED, LSD, df= 18, $P > 0.05$, SE = 12.14, $n = 40$) and adults (PROC MIXED, LSD, df = 21, $P > 0.05$, SE = 15.5, $n = 40$). Least squares means within a column followed by the same lowercase letter are not significantly different between insecticides for knockout time for nymphs (PROC MIXED, LSD, df = 18.27, $P > 0.05$, SE = 157.83, $n = 40$) and adults (PROC MIXED, LSD, df = 21, $P > 0.05$, SE = 134.88, $n = 40$). Least squares means within a column followed by the same lowercase letter are not significantly different between insecticides for percentage of mortality after knockout at feeding for nymphs (PROC MIXED, LSD, df = 12, $P > 0.05$, SE = 10.64, $n = 40$) and adults (PROC MIXED, LSD, df = 9, $P > 0.05$, SE = 4.03, $n = 40$). Means within a row followed by the no. are not significantly different between developmental stages for a single insecticide for feeding time (Student's *t*-test, df = 18, $P > 0.05$, SE = 13.93, $n = 40$), knockout time after feeding (Student's *t*-test, df= 18, $P > 0.05$, SE = 146.82, $n = 40$), and percentage of mortality after knockout at feeding (Student's *t*-test, df = 18, $P > 0.05$, SE = 8.06, $n = 40$).

of mortality after knockout was significantly higher for this compound than for all other treatments except for dicrotophos (Table 4). Knockout time was significantly greater for nymphs compared with adults for acetamiprid and dicrotophos, and percentage of mortality after knockout was significantly higher for nymphs for these insecticides and thiamethoxam.

Factorial analysis revealed that there was a significant insecticide effect for feeding time for *N. viridula* nymphs ($F = 4.33$; df = 6, 42; $P = 0.0017$) but not for adult females ($F = 1.71$; df = 6, 42; $P = 0.1426$) feeding on insecticide-contaminated gel-food. Feeding time was significantly lower for nymphs feeding on insecticides, except for dicrotophos, compared with those feeding on untreated food (Table 5). There was a significant insecticide effect for percentage of mortality 2 d after treatment for *N. viridula* nymphs ($F = 87.5$; df = 6, 42; $P = 0.0001$) and adults ($F = 88.67$; df = 6, 42; $P = 0.0001$) feeding on contaminated gel-food. Both nymphs and adults became incapacitated upon feeding on any insecticide, except indoxacarb (Table 5). This pattern of knockout at feeding was later reflected in percentage of mortality, which was significantly higher for insects feeding on contaminated food compared with those feeding on untreated food (Table 5). Although feeding acetamiprid-contaminated food resulted in knockout at feeding, percentage of mortality was significantly lower for insects feeding on acetamiprid compared with the other four insecticides. Feeding on indoxacarb-contaminated food did not result in knockout or mortality for either nymphs or adults.

For *T. pennipes*, factorial analysis revealed that there was a significant insecticide effect for feeding time ($F = 3.52$; df = 5, 30; $P = 0.0127$) when individuals fed on insecticide-treated sugar water. There was no significant sex effect ($F = 0.03$; df = 1, 30; $P = 0.8683$) and no insecticide and sex interaction ($F = 0.15$; df = 5, 30; $P = 0.9799$) for feeding time. Therefore, the four insects (two males and two females) in a block were

Table 5. Least squares means for percentage of feeding time and percentage of mortality 2 d after treatment for *N. viridula* nymphs and adults feeding on food contaminated with acetamiprid, cyfluthrin, dicrotophos, indoxacarb, oxamyl, and thiamethoxam

Insecticide	μg/ml	Feeding time (s)		% knockout at feeding[a]		% mortality 2 d after treatment	
		Nymphs	Adults	Nymphs	Adults	Nymphs	Adults
Acetamiprid	577	11.8bc	19.95a	100	100	75.0b	50.0b
Cyfluthrin	346	7.83c	20.61a	100	100	100.0a	100.0a
Dicrotophos	3,806	19.79ab	26.07a	100	100	100.0a	100.0a
Indoxacarb	1,038	15.48bc	20.84a	0	0	0c	0c
Oxamyl	2,883	15.03bc	18.48a	100	100	100.0a	100.0a
Thiamethoxam	715	10.29c	27.89a	100	100	100.0a	100.0a
Control		28.55a	28.70a	0	0	0c	0c

For nymphs, least squares means within a column followed by the same lowercase letter are not significantly different between insecticides for feeding time (PROC MIXED, LSD, df = 48.3, $P > 0.05$, SE = 3.42, $n = 16$). Least squares means within a column followed by the same lowercase letter are not significantly different between insecticides for mortality 2 d after treatment (PROC MIXED, LSD, df = 42, $P > 0.05$, SE = 0.05, $n = 16$). For adults, least squares means within a column followed by the same lowercase letter are not significantly different between insecticides for feeding time (PROC MIXED, LSD, df = 27.4, $P > 0.05$, SE = 4.0, $n = 16$). Least squares means within a column followed by the same lowercase letter are not significantly different between insecticides for mortality 2 d after treatment (PROC MIXED, LSD, df = 42, $P > 0.05$, SE = 0.05, $n = 16$).

[a] For information only.

Table 6. Least squares means for percentage of feeding time and percentage of mortality 2 d after treatment for *T. pennipes* adults feeding on food contaminated with acetamiprid, cyfluthrin, dicrotophos, indoxacarb, oxamyl, and thiamethoxam

Insecticide	µg/ml	Feeding time (s)	% knockout at feeding[a]	% mortality 2 d after treatment
Acetamiprid	577	2.48b	100	91.67a
Cyfluthrin	346	1.63b	100	100.0a
Dicrotophos	3,806	10.12a	100	100.0a
Indoxacarb	1,038	15.98a	0	100.0a
Oxamyl	2,883	7.98ab	100	100.0a
Thiamethoxam	715	6.17ab	100	100.0a
Control			0	0b

Least squares means within a column followed by the same lowercase letter are not significantly different between insecticides for feeding time (PROC MIXED, LSD, df = 12, $P > 0.05$, SE = 4.68, $n = 12$). Least squares means within a column followed by the same lowercase letter are not significantly different between insecticides for mortality 2 d after treatment (PROC MIXED, LSD, df = 12, $P > 0.05$, SE = 4.46, $n = 12$).

[a] For information only.

considered to be four random samples. Because there was no significant insecticide and sex interaction in this analysis, we conclude that the significance detected in preliminary analyses that included the control was promoted solely by the difference between males and females in the controls. Feeding times were significantly lower for adults feeding on cyfluthrin and acetamiprid compared with those feeding on dicrotophos and indoxacarb (Table 6). Feeding times for thiamethoxam and oxamyl were between these two groups of insecticides. All insecticides except indoxacarb caused *T. pennipes* adults to be knocked out at feeding. Feeding time was significantly reduced for adults feeding on insecticide-treated food for all treatments combined (7.39 ± 1.16) compared with those feeding on control food (39.49 ± 6.86) ($t = 4.62$, df = 11.6, SE = 4.91).

Factorial analysis revealed that there was a significant insecticide effect for percentage of mortality data ($F = 141.0$; df = 6, 33; $P = 0.0001$) for *T. pennipes* adults feeding on insecticide-treated sugar water 2 d after treatment. There was no significant sex effect ($F = 1.0$; df = 1, 33; $P = 0.3246$) and insecticide and sex interaction ($F = 1.0$; df = 6, 33; $P = 0.4418$) for percentage of mortality. Therefore, the four insects (two males and two females) in a block were again considered to be four random samples. All the insecticides were highly toxic to this parasitoid in these tests (Table 6).

Discussion

The combination of residual and oral toxicity studies provided valuable information on the overall susceptibility of the pest stink bug *N. viridula* to each of the insecticides. Dicrotophos, acetamiprid, and thiamethoxam were the only insecticides that exhibited residual activity and feeding activity in both oral toxicity tests. Of these three insecticides, dicrotophos was the most toxic to *N. viridula.* This toxicity was based on the finding that residual toxicity of dicrotophos was higher than that of the other two insecticides and that dicrotophos acted more quickly than acetamiprid. Also, although *N. viridula* were knocked down upon feeding on food containing these three toxicants, they were only able to recover when acetamiprid was the insecticide that was ingested. The dual residual and oral activity of dicrotophos against *N. viridula* may account for the reliable effectiveness of this insecticide in controlling this pest in producers' fields. Both oxamyl and cyfluthrin exhibited excellent residual activity and feeding activity when feeding on insecticide-contaminated food. Feeding on food with residues of these two insecticides reduced feeding time of stink bugs, but it did not result in mortality. In feeding tests, oxamyl acted more quickly than cyfluthrin, which may translate into less feeding damage on cotton bolls in the field. However, the ability of cyfluthrin to knockdown stink bugs may increase the susceptibility of these stink bugs to predators in the field and indirectly reduce feeding damage on bolls. A reduction in feeding time occurred with all the insecticides to varying degrees within and between the two feeding tests. Therefore, it would be interesting to examine whether there is a relationship between reduction in feeding time and amount of boll damage in the field.

Overall, our results are consistent with previous residual, topical, and field studies conducted on *N. viridula* with dicrotophos, cyfluthrin, oxamyl, and thiamethoxam (Greene et al. 2001, 2003; Fitzpatrick et al. 2002a,b; Greene and Capps 2003; López et al. 2003; Willrich et al. 2004a). Our study is the first one to demonstrate that each of these four insecticides also have ingestion activity against *N. viridula* and inhibit feeding by this pest.

In our study, indoxacarb exhibited no tarsal contact or direct ingestion activity against *N. viridula*, and it has previously been reported to have no impact on this pest in topical bioassays (Greene et al. 2001). However, we observed that indoxacarb reduced feeding time of *N. viridula* nymphs and adults and thus seems to make the stink bugs sick enough to stop feeding. Unfortunately, if the stink bugs are inhibited from feeding on indoxacarb, they also may not be able to feed long enough to ingest the quantity of toxin necessary to kill them.

Susceptibility of *E. servus* nymphs and adults to the same six insecticides used in this current study was determined earlier in similar residual and feeding experiments (Tillman and Mullinix 2004). Generally, the impact of these insecticides was very similar for *E. servus* and *N. viridula.* The exception was cyfluthrin which was very toxic to *N. viridula* but had little impact on *E. servus* in residual toxicity tests.

Our residual and oral toxicity experiments provide the first information on the susceptibility of *T. pennipes* to insecticides. Dicrotophos, oxamyl, cyfluthrin, and thiamethoxam were highly toxic to *T. pennipes* in both residual and feeding tests. Residues of these insecticides have been reported to be toxic to other natural enemies, especially predators, of stink bugs in cotton. Cyfluthrin residues were toxic to *Geocoris punctipes* (Say) (Tillman and Mulrooney 2001), and residues of this insecticide along with dicrotophos and oxamyl were very toxic to *Podisus maculiventris* (Say) (Tillman and Mullinix 2004). Residues of acetamiprid, which were moderately toxic to *T. pennipes,* also were moderately toxic to *P. maculiventris* (Tillman and Mullinix 2004). As in our study with *T. pennipes,* Studebaker and Kring (2001) determined that indoxacarb residues had no direct lethal impact on *Orius insidiosus* (Say). However, longevity and oviposition of *O. insidiosus* were adversely impacted when these predators were exposed to residues of this insecticide. Just as feeding on indoxacarb-treated sugar water was highly toxic to *T. pennipes,* feeding on indoxacarb-treated prey resulted in high mortality for *P. maculiventris* nymphs and adults (Tillman and Mullinix 2004). This insecticide possibly acts as a feeding inhibitor when consumed by *G. punctipes,* because females that previously fed on indoxacarb-treated prey were less likely to consume prey than females that previously fed on untreated prey (Tillman et al. 2001).

Each of the three new compounds, indoxacarb, acetamiprid, and thiamethoxam, developed to kill plant-feeding pests, was highly toxic to *T. pennipes* feeding on sugar water containing these compounds. Therefore, it cannot be assumed that an insecticide with ingestion activity against pests will have little impact on parasitoids and predators. Evaluation of the effects of ingesting insecticides is essential to understanding the potential impact these insecticides could have on natural enemies when these compounds are applied to cotton fields. Natural enemies may ingest residues of insecticides during any of the following scenarios in the field: 1) feeding on plant tissue, pollen, or nectar contaminated with insecticide residues; 2) drinking rain water contaminated by insecticides; 3) feeding on systemic insecticides while sucking on plant sap; 4) preying on insects that have eaten foliage covered with insecticide residues; or 5) preying on insects covered with insecticide residues. In a unique study, Boyd and Boethel (1998) demonstrated the transfer of toxicity of certain insecticides from a prey species that consumed insecticides to *G. punctipes.* In these tests, most of the newer compounds, such as spinosad, were more selective than older compounds, such as methyl parathion. Other studies have demonstrated that thiamethoxam increased developmental time and reduced body weight of *Podisus nigrispinus* (Dallas) when nymphs were caged on cotton plants after application of the compound to the soil (Torres et al. 2003). Another novel study examining the impact of insecticides on *P. maculiventris* via ingestion was conducted by allowing individuals of this predator to drink water contaminated by insecticides (Mohaghegh et al. 2000). Fourth instars and adult females suffered the highest mortality when they ingested methomyl compared with the other insecticides. Recently, Vandekerkhove and De Clercq (2004) reported that nymphs and adults of *N. viridula* were more susceptible to an encapsulated formulation of the pyrethroid lambda-cyhalothrin than nymphs of *P. maculiventris* both by ingestion and contact exposure.

Studies comparing susceptibility of insecticides between natural enemies and their prey are vital for evaluating insecticide selectivity to natural enemies. Tillman and Mullinix (2004) determined that insecticide selectivity to *P. maculiventris* was detected for only acetamiprid when the same six insecticides used in this current study were examined. In a field test, though, acetamiprid controlled pear psylla, *Cacopsylla pyricola* (Foerster), but it also disrupted natural enemy populations (Brooks et al. 2004). In cotton field trials, acetamiprid and thiamethoxam provided adequate control of the cotton aphid, *Aphis gossypii* (Glover) (Kilpatrick et al. 2004), but thiamethoxam had an adverse impact on populations of two predators, *G. punctipes* and *Solenopsis invicta* Buren. Both the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), and *G. punctipes* responded very similarly to indoxacarb in topical, tarsal contact, and plant feeding toxicity studies in the laboratory (Tillman et al. 2001). In cotton fields, indoxacarb significantly reduced population densities of *Lygus* spp. and *O. insidiosus* (Muegge and Payne 2001). Oxamyl, cyfluthrin, and encapsulated methyl parathion were evaluated in cotton field plots throughout the season for control and boll weevils and their impact on natural enemies (Parker and Huffman 1997). Generally, cyfluthrin and oxamyl were equally effective in controlling boll weevils, whereas methyl parathion was less effective in controlling this pest compared with the other two insecticides. However, each of the three insecticides equally reduced population densities of natural enemies in these fields.

In this study, susceptibility of the tachinid parasitoid *T. pennipes* to insecticides was higher than or equal to that of adults of the stink bug pest *N. viridula* in residual and oral experiments. *T. pennipes* was slightly more susceptible than *N. viridula* to acetamiprid and thiamethoxam in residual tests and to acetamiprid in feeding tests. Feeding on indoxacarb-treated food was basically nontoxic *to N. viridula* but highly toxic to *T. pennipes.* This difference in susceptibility to this insecticide was probably not because of the difference in size between the insect species, because there was no impact on either stink bug nymphs or adults. It seems that the two species were responding differently in some way when feeding on food contaminated by indoxacarb. There may be species differences in feeding physiology or in bioactivation of the compound once ingested. Nevertheless, because insecticide selectivity to *T. pennipes* was not detected in these studies, it is extremely important to conserve *T. pennipes* in cotton fields by providing refuges for the parasitoid and by applying these insecticides for control of southern green stink bugs only when the pest reaches economic threshold.

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